Our ref: KON-1870 Client's ref: P6388-001-0000 (US)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of: Eiichi UEDA, et al : Art Unit: 1618

Appln. No.: 10/824,095 : Examiner: M.J. Perreira

Filed: April 13, 2004 :

For: LIPOSOME-CONTAINING RADIOGRAPHIC CONTRAST MEDIUM AND PREPARATION METHOD THEREOF :

CONFIRMATION #6153

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DECLARATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

S i r:

I, Akihisa NAKAJIMA, hereby declare and say as follows:

1. I am one of the named Inventors in this Application.

2. I graduated from Osaka University in March of 1987 with a Masters Degree in Synthetic Chemistry. Since April of 1987, I have been employed by Konica Corporation, the Assignee of the above-identified Application and have specifically been engaged

in the research and development of photographic material supports.

3. I submitted a Declaration dated November 17, 2008 in this Application and I am aware that the Examiner commented on the Declaration of November 17, 2008 in an Office Action dated December 12, 2008.

4. I am aware that the Examiner took the position that Otake (US 2004/009976) teaches trapping efficiencies as high as 45% and the Declaration of November 17, 2008 provided data for only one phospholipid, DPPC, which did not show the highest trapping efficiency for the different phospholipids taught in Otake in Fig. 8. The trapping efficiency of a liposome depends on both the phospholipid used to make the liposome and the substance to be encapsulated by a phospholipid. In other words, a proper side-by-side comparison must keep constant both the phospholipid and the encapsulated substance.

5. The hydration of the encapsulated substance is an important factor in the trapping efficiency of liposome. Figure 8 of Otake was obtained from the results of Example 4 of Otake. Example 4 of Otake used glucose as the encapsulated substance. Glucose is a highly water soluble substance and should have a

larger trapping efficiency than iohexol which has a smaller water solubility than glucose. Iohexol was used as the encapsulating substance in the November 17, 2008 Declaration.

6. In order to respond to the Examiner's comments about the data in Figure 8 of Otake, tests have been performed to repeat Example 4 of Otake with glucose for the encapsulated substance and dioleoylphosphatidylcholine (DOPC) as the phospholipid. Comparative tests have also been performed with iohexol to demonstrate that trapping efficiency is affected by the encapsulated substance.

7. I am also aware that the Examiner has stated that the test data provided in the Declaration of November 17, 2008 providing a comparison of PEGylated liposomes (phospholipid modified with a polyalkylene oxide) of this Application to the liposomes taught in Otake (US 2004/0099976) was not enough data. Therefore, additional tests have been performed to provide more data.

8. To show that the trapping efficiency of a liposome depends on the substance to be encapsulated by a phospholipid, four new Samples 6-1 through 6-4 were made. Each of the samples were prepared with DOPC as the phospholipid and the concentration of

the phospholipid to the amount of added water was 20 mM. Sample 6-1, in which the substance encapsulated was glucose, was prepared as described in Example 4 of Otake. Sample 6-2 was prepared as Sample 6-1, except the substance to be encapsulated was iohexol in an amount of 647 mg/ml, having an iodine content of 300 mgI/mL. Sample 6-3 was prepared in the same manner as Sample 6-1, except no ethanol was used. Sample 6-4 was prepared in the same manner as Sample 6-2 except no ethanol was used. The trapping efficiency is reported in Table 6.

9. As can be seen in Table 6, the trapping efficiency of Sample 6-2, containing iohexol, was much lower than that of the Sample 6-1 containing the glucose solution by a factor of 1.5 to 2. The test results reported in Table 6 demonstrate that the trapping efficiency of a liposome depends on the substance to be encapsulated by a phospholipid.

10. To provide more data relating to the claims, a new set of liposomes were prepared using three different phospholipids. The concentration of the phospholipids with respect to the amount of added water was 20mM. The samples were prepared as follows:

a. Sample 7-1 was prepared with DOPC as the phospholipid in the same manner as described in Example 4 of Otake except

that no ethanol was used and that the substance to be encapsulated was iohexol in an amount of 647 mg/ml, having an iodine content of 300 mgI/mL.

b. Sample 7-2 was prepared in the same manner as Sample 7-1, except that it also included DSPE-020CN, which is a phospholipid modified with a polyalkylene oxide, available from NOF Corporation.

c. Sample 7-3 was prepared in the same manner as Sample 7-1, except that it also included pluronic F-88, a compound containing a polyoxyalkylene group, available from ADEKA Co.

d. Sample 7-4 was prepared in the same manner as Sample 7-1, except that DPPC was the phospholipid used instead of DOPC.

e. Sample 7-5 was prepared in the same manner as Sample 7-2, except that DPPC was the phospholipid used instead of DOPC.

f. Sample 7-6 was prepared in the same manner as Sample 7-3, except that DPPC was the phospholipid used instead of DOPC.

g. Sample 7-7 was prepared in the same manner as Sample 7-1, except that DSPC was the phospholipid used instead of DOPC.

h. Sample 7-8 was prepared in the same manner as Sample 7-2, except that DSPC was the phospholipid used instead of DOPC.

i. Sample 7-9 was prepared in the same manner as Sample 7-3, except that DSPC was the phospholipid used instead of DOPC.

11. The proportion (weight percentage of iodine compound in the vesicle based on total iodine compound) of each of the samples is reported in Table 7 attached to the Declaration.

12. As can be seen in Table 7, the amount of iodine in the vesicle in Samples 7-2 and 7-3 was greater than the amount of iodine in the vesicle of Sample 7-1 by a factor of about 1.5 to 2. Also noted in Table 7, the amount of iodine in the vesicle in Samples 7-5 and 7-6 was greater than the amount of iodine in the vesicle of Sample 7-4 by a factor of about 2. Additionally, Table 7 also shows that the amount of iodine in the vesicle in Samples 7-8 and 7-9 was greater than the amount of iodine in the vesicle of Sample 7-7 by a factor of about 1.5. It is surprising and unexpected that the amount of iodine in Samples 7-2, 7-3, 7-5, 7-6, 7-8 and 7-9 is greater than the amount of iodine in Samples 7-1, 7-4 and 7-7.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 USC 1001 and that such

willful false statements may jeopardize the validity of the application or any patent issued thereon.

Akihisa Nakajima
Akihisa NAKAJIMA

Dated: This 18th day of February, 2009.

DCL/cmj/mr

Attached: Table 6
Table 7

Table 6

sample	method of preparing a liposome	substance to be encapsulated	Trapping efficiency(%)
6-1	the Example 4 of Otate	glucose	45
6-2	the Example 4 of Otate	647 mg/ml of iohexol (300mgI/mL)	19
6-3	the Example 4 of Otate, except no ethanol was used	glucose	24
6-4	the Example 4 of Otate, except no ethanol was used	647 mg/ml of iohexol (300mgI/mL)	15

Table 7

Sample	method of preparing a liposome	substance to be encapsulated	Lipids	substance to be mixed with a phospholipid	propotion (weight percentage of iodine compound included in vesicles based on total iodine compound)	Remarks
7-1	the Example 4 of Otake, except no ethanol was used	647 mg/ml of iohexol (300mgI/mL)	DOPC	none	15%	Comparative
7-2	the Example 4 of Otake, except no ethanol was used, further a phospholipid modified with a polyalkylene oxide was mixed	647 mg/ml of iohexol (300mgI/mL)	DOPC	a phospholipid modified with a polyalkylene oxide (DSPE-020CN, available from NOF CORPORATION)	40%	Inventive
7-3	the Example 4 of Otake, except no ethanol was used, further a compound containing a polyoxyalkylene group was mixed	647 mg/ml of iohexol (300mgI/mL)	DOPC	a compound containing a polyoxyalkylene group (pluronic F-88, available from ADEKA Co.)	25%	Inventive
7-4	the Example 4 of Otake, except no ethanol was used	647 mg/ml of iohexol (300mgI/mL)	DPPC	none	13%	Comparative
7-5	the Example 4 of Otake, except no ethanol was used, further a phospholipid modified with a polyalkylene oxide was mixed	647 mg/ml of iohexol (300mgI/mL)	DPPC	a phospholipid modified with a polyalkylene oxide (DSPE-020CN, available from NOF CORPORATION)	25%	Inventive
7-6	the Example 4 of Otake, except no ethanol was used, further a compound containing a polyoxyalkylene group was mixed	647 mg/ml of iohexol (300mgI/mL)	DPPC	a compound containing a polyoxyalkylene group (pluronic F-88, available from ADEKA Co.)	20%	Inventive
7-7	the Example 4 of Otake, except no ethanol was used	647 mg/ml of iohexol (300mgI/mL)	DSPC	none	12%	Comparative
7-8	the Example 4 of Otake, except no ethanol was used, further a phospholipid modified with a polyalkylene oxide was mixed	647 mg/ml of iohexol (300mgI/mL)	DSPC	a phospholipid modified with a polyalkylene oxide (DSPE-020CN, available from NOF CORPORATION)	20%	Inventive
7-9	the Example 4 of Otake, except no ethanol was used, further a compound containing a polyoxyalkylene group was mixed	647 mg/ml of iohexol (300mgI/mL)	DSPC	a compound containing a polyoxyalkylene group (pluronic F-88, available from ADEKA Co.)	17%	Inventive